1st SEMIANNUAL *REPORT ON*

CONTRACT NO DA92-557-FEC-35779

INCLUSIVE DATES February 1963 *TO* 31 July 1963

SUBJECT OF INVESTIGATION

THE BIOLOGICAL SIGNIFICANCE AND CHEMISTRY
OF
A PROTEASE INHIBITOR NEWLY ISOLATED
FROM
ANIMAL TISSUES

RESPONSIBLE INVESTIGATOR

Dr. Hideo Hayashi

Professor of Pathology and Head of the First Department, Kumamoto University Medical School, Kumamoto, Japan

U.S. Army Research & Development Group (9984) (Far East)
Office of the Chief of Research and Development
United States Army
APO 343

D-I-S-T-R-I-B-U-T-I-O-N

The distribution of this report as made by USA R&D Gp (FE) is as follows:

Army Research Office, 3045 Columbia Pike, Arlington 4, Virginia. ATTN: Chief Research Programs Office (4)

Army Attache, American Embassy, Tokyo, Japan (1)

Office of Primary Scientific Liaison (1)
U.S. Army Medical Research & Development Command
ATTN: Chief, Preventive Medicine Research Branch
Washington 25, D. C.

~~**Offices of Scientific Cognizance**~~ ( )

APPENDIX "A"

1. Resume of the present progress:

As described in a final report, March, 1963, a specific protease inhibitor of polypeptide nature was isolated from healing skin site of Arthus inflammation and extensively purified as fibrous substance (Fig. I). The inhibitor was solved in buffer and its solution was equilibrated with various buffers of desired pH by means of dialysis; and it received a paper electrophoretic analysis. There was revealed only one spot positive with amidoschwarz 10 B staining, showing successful purification of the inhibitor (Fig. II). A Grassmann's apparatus was used. This inhibitor inactivated particular SH-protease of cutaneous Arthus inflammation and papain, but had no effect on trypsin or chymotrypsin.

Mobilities as a function of pH were computed from a pH mobility curve according to Kunkel and Tisselius; and the isoelectric point of this inhibitor seemed to be around 6.6, as shown in Fig. III.

Fig. I. Photograph showing fibrous inhibitor (1310 IE/E_{280}). x 800.

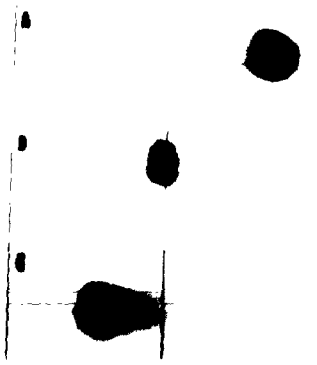
Fig. II. Paper electrophoresis of inhibitor. Inhibitor concentration: 2.67 mg. N/ml. (0.01 ml. used). 240 min., 2.15 volts per cm. A: Na acetate buffer pH 3.85, 0.1 M. B: Phosphate buffer, pH 6.8, 0.1 M (also tested in 420 min., 4.4 volts per cm; and the same results obtained). C: Barbiturate buffer, pH 8.6, 0.1 M.

Fig. III. Effect of pH on mobility of inhibitor. Mobility in $cm^2\ sec^{-1}\ volt^{-1}$.

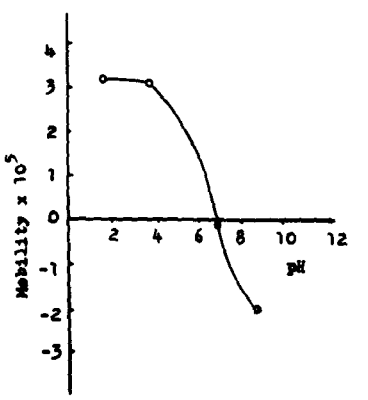
2. Research activity for the next half:

The above inhibitor receives further chemical analysis, for instance, by an ultracentrifugation and amino acids-analyser. Crystallization of this inhibitor is completed; and its biological action on various types of inflammation is assayed. Also, the inflammatory vascular permeability factor and its anti-substance, discussed in a final report, are purified and their dynamic relationship in the vascular phenomenon in inflammation is studied. Furthermore, the relationship between the antiprotease and antipermeability factor is searched for essential understanding of inflammation. All these substances were pointed out by us.

(Fig. 1)



(Fig. 2)



(Fig. 3)